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## Genetic and genomic studies in ovine mastitis

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### ABSTRACT

Mastitis is a strong financial and animal welfare concern in both dairy and meat-producing sheep. In this review article, we summarized recent advances in research on genomic of mastitis resistance in sheep. Heritability estimates for mastitis-related traits such as somatic cell scores or milk bacteriological counting have confirmed a genetic basis for mastitis resistance. Recent outputs from genomic-based studies comprising genome-wide associations, the identification of one causal mutation as well as gene expression studies have highlighted regions of the genome, and possible genes and mechanisms underlying the resistance trait. Part of genomic regions was common among breeds and populations, and have testified to the partial sharing of mastitis-related genetic mechanisms between different distant dairy sheep populations. Accumulating genetic data, however, have underlined the polygenic nature of the trait and the complexity of the resistance phenotype. Both quantitative and genomic analyses have further revealed trade-offs and synergies under genetic control between mastitis resistance and other efficiency (milk production) and resilience related traits (udder type traits and metabolic disease). We have also reported how phenotypic, pedigree and genomic information has been used in sheep breeding, the aim being to improve the animals' health and welfare, the hygienic quality of milk products and overall efficiency and resilience.

### 1. Introduction

Genetics of mastitis has been studied more recently in dairy sheep (Baro et al., 1994; El-Saied et al., 1999; Barillet et al., 2001; Serrano et al., 2003) than in dairy cattle where it has been increasingly well documented since the 1980s (Emanuelson and Philipsson, 1984; Emanuelson et al., 1988; Mrode and Swanson, 1996; Heringstad et al., 2000; Detilleux, 2002; Rupp and Boichard, 2003). In general, relevant studies have focused on dairy sheep, with few data produced in meat or wool production sheep populations.

First evidence that a host's response to mastitis was under genetic control, came from quantitative genetic studies and the estimation of genetic parameters for mastitis-related traits. The approach relies on the assumption that the trait is determined by a combination of animal (age, lactation stage, etc.), genetic (breed, line, etc.), and environmental factors, the latter including both microbiological (infection pressure, pathogen species and strain, etc.) and husbandry parameters (breeder practices regarding machine milking, sheepfold, etc.). No assumption had been made about the underlying genes and mechanisms. The heritability parameter quantifies the amount of variability in a given trait that is due to additive genetic values. It also allows the

estimation of genetic value for the animals (breeding value) and predicting to what extent a selection process can be efficient. These studies, however, require large data sets with phenotypic information and pedigree. Therefore, the most common mastitis-related trait used in ovine mastitis genetic studies is the somatic cell count (SCC). Indeed, somatic cell count can be easily measured in milk samples, as an indirect predictor of mammary infection, which is much more frequent than clinical mastitis in sheep (Bergonier et al., 2003).

Since 2009, the development of genomic methods and tools applied to sheep have contributed to enhance insights into the genetic control of processes governing host resistance to mastitis. The sheep genome was sequenced in 2007 (Jiang et al., 2014) and several low- to high-density ovine SNP (single-nucleotide polymorphism) chips have been developed as part of the International Sheep Genomics Consortium (ISGC; [www.sheepmap.org](http://www.sheepmap.org)) (Kijas et al., 2009). Availability of such tools has enabled genome scans to identify and fine map genomic regions associated with traits (QTL for Quantitative Trait Loci), the gene expression studies to highlight pathways and genes involved in the host's response to infection, and the first use of genome-wide information in sheep breeding.

This paper reviews the state of the art on the genetic control of

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**Table 1**  
Estimates of heritabilities for mastitis-related traits and genetic correlations with milk yield and mastitis-related traits in ewes.

Reference (Chronological order)	Sheep breed	Number of		Trait	Model	Heritability	Genetic correlations: trait and	
		Records	Ewes				Milk yield	Other mastitis traits
Baro et al., 1994	Churra	10,171 test-days	3,832	Somatic cell scores	Test-day	0.04	-0.37	-
El-Saied et al., 1999	Churra	3,231 lactation periods	2,379	Somatic cell scores	Lactation period	0.12	-0.15	-
Barillet et al., 2001	Lacaune	23,091 test-days	5,272	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.15	0.11	-
Ohmane et al., 2002a	Churra	1,962 lactation periods	1,111	Somatic cell scores	Lactation period	0.11	-0.17	-
Ohmane et al., 2002b	Churra	7,492 test-days	1,119	Somatic cell scores	Test-day	0.11	-0.36	-
Gonzalo et al., 2003	Churra	4,692 test-days	1,337	Somatic cell scores	Test-day	0.09 - 0.11	-	-
Rupp et al., 2003	Lacaune	273,975 test-days	94,445	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.13	0.18	SCS (2 <sup>nd</sup> lactation period): 0.93
Serrano et al., 2003	Manchega	131,947 test-days	45,499	Somatic cell scores (2 <sup>nd</sup> lactation period)	Lactation period	0.12	0.08	SCS (1 <sup>st</sup> lactation period): 0.93
		37,641 test-days	10,962	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.12	-0.12	-
		36,793 test-days	10,560	Somatic cell scores (2 <sup>nd</sup> lactation period)	Lactation period	0.19	-0.14	-
		29,358 test-days	8,379	Somatic cell scores (3 <sup>rd</sup> lactation period)	Lactation period	0.24	-0.15	-
Hamann et al., 2004	East Friesian	9,729 test-days	1,108	Somatic cell scores	Test-day	0.16	0.02	-
Legarra and Ugarte, 2005	Laxa	9,805 lactation periods	6,165	Somatic cell scores	Lactation period	0.13	-0.30	-
Barillet, 2007	Lacaune	121,283 lactation periods	121,283	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.15	0.15	-
Riggio et al., 2007	Valle del Belice	13,066 test-days	2,277	Somatic cell scores (1 <sup>st</sup> lactation period)	Test-day	0.14	0.23	-
Barillet et al., 2009	Manech Red Face	763,458 test-days	58,378	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.10	0.21	-
Casi et al., 2010	Sarda, Sarda × Lacaune	1,682 test-days	1,587	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.26 - 0.28	-	-
Riggio et al., 2010	Valle del Belice	8,843 test-days	1,120	Somatic cell scores	Test-day	0.09	-	-
				Infection status (bacteriological examination)	Threshold animal	0.09	-	SCS infected / uninfected ewes: 0.81 / 0.51
					Test-day	0.03	-	InfS / SCS uninfected ewes: 0.81, 0.62
					Test-day	0.10	-	InfS / SCS infected ewes: 0.51 / 0.62
De la Fuente et al., 2011	Churra	10,189 test-days	3,977	Somatic cell scores	Test-day	0.09	-	-
Tolone et al., 2013	Valle del Belice	17,843 test-days	2,040	Somatic cell scores	Test-day	0.09	-	InfS: 0.93
				Infection status (bacteriological examination)	Threshold animal	0.09	0.59	SCS: 0.93
				Infection status (bacteriological examination)	Threshold animal	0.02	-	InfS by cns / by Str.: 0.92 / 0.36
Tolone et al., 2016	Valle del Belice	5,305 lactation periods	2,350	Infection status by coagulase-negative staphylococci (bacteriological examination)	Threshold animal	0.02	-	InfS / InfS by Str: 0.92 / 0.24
	Valle del Belice			Infection status by <i>Streptococcus</i> spp. (bacteriological examination)	Threshold animal	0.09	-	InfS / InfS by cns: 0.36 / 0.24
Banos et al., 2017	Chios	2,436 samples	609	Somatic cell scores (1 <sup>st</sup> and 2 <sup>nd</sup> lactation period)	Test-day (week)	0.11	-0.12	CMT / TBC / Dev c/m: 0.77 / 0.56 / 0.35
				Scores of California Mastitis Test (1 <sup>st</sup> and 2 <sup>nd</sup> lactation periods)	Test-day	0.12	-0.12	CMT / TBC / Dev c/m: 0.77 / 0.21 / 0.36
				Total bacterial counts (1 <sup>st</sup> and 2 <sup>nd</sup> lactation periods)	Logit function	0.09	-0.11	SCS / CMT / Dev c/m: 0.56 / 0.21 / 0.35
				Development of clinical mastitis (1 <sup>st</sup> and 2 <sup>nd</sup> lactation periods)	Linear animal	0.18	-0.09	SCS / CMT / TBC: 0.35 / 0.36 / 0.35
O'Brien et al., 2017	Belclare, Charollais, Suffolk, Texel, Vendeen	3,378 examinations	3,378	Scores of California Mastitis Test	Lactation period	0.22	0.12	StaphC: 0.72
Allain et al., 2018	Lacaune	-	377,945	Somatic cell scores (1 <sup>st</sup> lactation period)	Lactation period	0.18	0.31	SCS: 0.72
			518	Staphylococcal counts (1 <sup>st</sup> lactation period)	Lactation period	0.18	0.31	SCS: 0.72

(continued on next page)

Table 1 (continued)

Reference (chronological order)	Sheep breed	Number of		Trait	Model	Heritability	Genetic correlations: trait and	
		Records	Ewes				Milk yield	Other mastitis traits
McLaren et al., 2018	Texel	3,410 samples	2,957	Average somatic cell scores of samples from both mammary glands	Animal model	0.08 - 0.11	-	Sum CMT: 0.76 - 0.96 Max CMT: 0.79 - 0.98
		3,539 samples		Sum of scores of California Mastitis Test in samples from both mammary glands		0.09 - 0.11	-	Av SCS: 0.76 - 0.96 Max CMT: 0.99
		3,529 samples		Maximum score of California Mastitis Test in samples from both mammary glands		0.07 - 0.08	-	Av SCS: 0.79 - 0.98 Sum CMT: 0.99

SCS: Somatic Cell Score, InfS: Infection Status, cns: coagulase-negative *Staphylococci*, Str.: *Streptococci*, CMT: scores of California Mastitis Test, TBC: Total Bacterial Counts, Dev c/m: Development of clinical mastitis, StaphC: *Staphylococcal* Counts, Sum CMT: Sum of scores of California Mastitis Test in samples from both mammary glands, Max CMT: Maximum score of California Mastitis Test in samples from both mammary glands, Av SCS: Average Somatic Cell Scores of samples from both mammary glands.

\* Results obtained by Banos et al. (pre-print on the BioRxiv platform, doi: <https://doi.org/10.1101/577015>) that used the same samples as in Banos et al. (2017).

resistance to mastitis, with primary reference to sheep. Genetic parameters for different mastitis-related traits are presented and genetic associations with other traits of interest are discussed. The paper then reports on recent outputs from genomic-based studies comprising QTL detection studies and the identification of causal mutations as well as gene expression studies. Finally, it considers how information using genome-wide information (or not) is currently used in sheep breeding with some discussion on future prospects.

## 2. Genetic parameters of ovine mastitis

### 2.1. Genetic parameters for mastitis-related traits

For three decades, published literature on genetic parameters of ovine mastitis in dairy sheep has been accumulating, as reported in earlier reviews (Carta et al., 2009; Rupp and Foucras, 2010; Riggio and Portolano, 2015). The most common mastitis-related traits used in genetic studies is a logarithmic transformation of somatic cell count termed 'Somatic Cell Score' (SCS) (Ali and Shook, 1980). Heritabilities for somatic cell scores (lactation period average or repeated test day records) have been reported in sheep, as shown in Table 1. Heritability estimates are low and range from 0.04 to 0.28 with a weighted average of 0.163 (Table S1 for the explanation of the calculation) for various dairy sheep breeds, including Chios (Greece), Churra (Spain), East Friesian (Germany), Lacaune (France), Latxa (Spain), Manchega (Spain), Manech Red Face (France), Sarda (Italy) and Valle del Belice (Italy). These heritabilities showed that genetic variation existed for this trait, although to a lesser extent than for production traits with heritabilities ranging from 0.30 to 0.60. The California Mastitis Test score is another less used indirect mastitis-related trait based on milk somatic cells. This test consists of the addition in the milk of a reagent composed of a detergent and a pH indicator. When mixed with the milk, the reagent reacts and forms a viscous gel, which can be scored using a range of milk color and viscosity, therefore indicating the number of somatic cells and the level of inflammation. Only one study in dairy sheep (Banos et al., 2017) estimated a heritability for this trait (0.12), which is in the same range as for somatic cell score. However, both somatic cell scores and results of the California Mastitis Test are two indirect measures for mastitis, which reflect the degree of inflammation in the mammary gland rather than infection status. The trait, therefore, combines the measure of severity of infection and intensity of the host's response. Direct measures of mastitis, e.g., the occurrence of clinical mastitis based on examinations of veterinarians have been very little studied in dairy sheep, because of the small frequency of the disease (Bergonier et al., 2003) and the difficulty to implement it on a large scale. Heritability of this binary trait (presence/absence of clinical mastitis) has been estimated at 0.18 in the Chios breed (Banos et al., 2017), which is also in the same range as for somatic cell scores.

Very recently, studies in meat-producing sheep breeds have estimated genetic parameters for mastitis-related traits, as it is a strong financial and animal welfare concern (Conington et al., 2008). Heritability of occurrence of clinical mastitis was estimated at 0.04 in an Irish population including Belclare, Charollais, Suffolk, Texel and Vendeen breeds (O'Brien et al., 2017). In the Texel breed, somatic cell scores and results of California Mastitis Test estimates of heritability ranged from 0.07 to 0.11 (McLaren et al., 2018). Although these heritabilities are somewhat smaller than in dairy sheep, probably due, at least partly, to the recording scheme (only one examination per animal), they have confirmed that, as expected, genetic control for mastitis also exists in those meat-producing sheep breeds.

During the last decade, genetic studies have focused on bacteriological analyses of milk samples, in order to better quantify the intramammary bacterial pressure in dairy sheep. The most widely used technique in the dairy sheep literature is a count of bacterial colonies in milk samples after incubation on agar plates. This conventional technique leads to a binary trait of infection status (ewe considered as

infected if over 5 colony-forming-units per 10  $\mu$ L of milk of one bacterial species would be recovered). Estimates of heritability of this trait termed 'infection status' ranged from 0.02 to 0.09 (Table 1) in the Valle del Belice breed (Riggio et al., 2010; Tolone et al., 2013, 2016) and seem to depend on the species of bacteria. One study (Banos et al., 2017) has estimated heritability of another trait based on bacteriological examination of milk samples, the total bacterial count, which refers to the number of viable bacteria per mL of milk. Heritability of this estimate is of the same order of magnitude as the infection trait (0.09), which is lower than heritabilities of the traits based on milk somatic cells. One last technique, recently employed in the French La-caune breed (Allain et al., 2018; Oget et al., 2019) has been based on amplification on staphylococcal DNA by qPCR in milk samples, representing the number of bacterial genomes in 10  $\mu$ L of milk. Estimate of heritability of this continuous trait was in the same range as somatic cell scores (0.18).

Genetic correlations have also been estimated (Table 1) between the various mastitis-related traits described above. The occurrence of clinical mastitis has been found to be positively correlated with somatic cell scores, results of California Mastitis Test and total bacterial counts (0.35 - 0.36) in Chios animals (Banos et al., 2017), suggesting a common genetic basis between development of disease and the various traits indirectly indicating mastitis. Genetic correlations between the two leucocyte-related traits (results of California Mastitis Test and somatic cell scores) ranged from 0.76 to 0.98 (Banos et al., 2017; McLaren et al., 2018), confirming the usefulness of the California Mastitis Test as an alternative to cell counting. Infectious status (conventional technique) and somatic cell scores have also been found to be highly correlated (0.51 to 0.93) (Riggio et al., 2010; Tolone et al., 2013), as did also total bacterial counts with somatic cell scores (0.56) (Banos et al., 2017) and staphylococcal DNA with somatic cell scores (0.72) (Allain et al., 2018). Milk bacteriological traits seemed then to be genetically close to the most used mastitis-related trait 'somatic cell score'. These reasonably high values have suggested a commonality of resistance mechanisms that lead to better resistance to either persistent intra-mammary infection (continuously high SCC) or acute clinical episodes with presence of pathogens in the udder, despite the fact that those forms of mastitis might be associated with various environmental conditions, pathogens, and status of the animal.

## 2.2. Genetic relationships of mastitis resistance with other traits

### 2.2.1. Relationships with milk production traits

Genetic correlation estimates between mastitis-related traits and milk yield trait in sheep are presented in Table 1. These are inconsistent across the various dairy breeds. Indeed, estimates were found to be negative (i.e., favorable) for Spanish and Greek breeds, with genetic correlations from  $-0.37$  for Churra to  $-0.09$  for Chios ewes, and positive (i.e., unfavourable) for German, Italian and French breeds, with genetic correlations 0.02 for East Friesian to 0.59 for Valle del Belice ewes. The weighted average of these estimates is 0.078 (Table S1). An explanation for these inconsistent results between the different breeds may simply be statistical reasons. Differences in genetic correlation estimates between Spanish and the other breeds can be due to lower selection pressure applied in the Spanish breeds, or to the fact that estimates in Spanish breeds were mainly obtained considering different lactations whereas, in the other breeds, they mostly considered only the first lactation. Some pleiotropic genes, for example the group-specific component (*GC*) gene, could explain the relation between udder health and production traits. This gene encodes the vitamin D-Binding Protein (DBP) which has multiple roles in immune defense and milk production, as found in cattle (Olsen et al., 2016). Another pleiotropic gene is the *suppressor of cytokine signalling2* (*SOCS2*) gene, which has been found to be associated with mastitis in sheep and also had an effect on growth and milk production (Rupp et al., 2015). It also can be due to a selection sweep, when selection for milk production has 'hitchhiked'

along with it a nearby allele on the chromosome that is associated with mastitis susceptibility. Other possible explanations for strong unfavorable genetic correlations are the biological competition between functions for energy and nutrients and the relationship between mastitis and udder type traits.

### 2.2.2. Relationships with udder type traits and milking ease

A few studies in dairy and meat-producing sheep have dealt with estimating genetic correlations between udder-type and mastitis-related traits (Legarra and Ugarte, 2005; Barillet, 2007; Casu et al., 2010; McLaren et al., 2018). Udder attachment was negatively correlated with mastitis (genetic correlations:  $-0.27$  to  $-0.42$ ), whereas long teats were favorable for mastitis (genetic correlations: 0.26 to 0.44), indicating that ewes with a pendulous udder or longer teats are at greater risk to develop mastitis. Moreover, teat angle has also been negatively correlated with mastitis (genetic correlations:  $-0.12$  to  $-0.55$ ). One study also has demonstrated the positive genetic relationship between mastitis resistance and milking ease (Allain et al., 2018). Indeed, genetic correlations of somatic cell scores or quantity of staphylococcal DNA in milk with milk flow traits ranged from 0.26 to 0.30 and from  $-0.04$  to  $-0.33$  with latency time and real milking time, respectively. These values confirmed that ewes with the higher relaxing ability of the teat muscle (able to quickly release milk) allowed more pathogens to enter into the mammary gland, potentially leading to mastitis.

### 2.2.3. Relationships with body, feet type and meat production traits

Genetic association of mastitis traits with body conformation, feet type, and meat production traits have been found to be close to zero. Indeed, the estimate of genetic correlation of somatic cell scores with litter size was at 0.001 for East Friesian ewes (Hamann et al., 2004). Estimates with morphological traits, including stature, rear legs, feet angle, rump width, and general body score, ranged from  $-0.06$  to 0.11, with high standard errors for Churra ewes (De la Fuente et al., 2011), as well as for meat-producing sheep breeds (O'Brien et al., 2017). These results indicate that selection for one of these traits would not affect susceptibility to mastitis of the individuals.

### 2.2.4. Relationship with resistance to other diseases

Mastitis is mainly caused by extracellular and facultative intracellular bacteria and the host's resistance probably involves a crucial role of the type-1 immune response. However, the control of parasites is predominately governed by a type-2 immune response. Also, there is evidence that different components of the immune response, including type-1 and type-2 responses, and resistance to various diseases, show null to antagonistic genetic correlation (Mouton et al., 1984; Pinard van der Laan, 2002; Crawley et al., 2005). In sheep, a link between mastitis resistance with resistance to gastrointestinal nematodes was reported in an experiment, in which sheep divergently selected for either high or low somatic cell counts received an oral challenge with *Haemonchus contortus* (Traoré et al., 2008). Whereas lambs showed highly variable response to challenge, there was no difference between the genetic lines, which suggested that genetic resistance to either mastitis or gastrointestinal nematodes were under independent genetic regulations.

The link of mastitis resistance to metabolic disease was addressed in an experiment, in which sheep divergently selected for either high or low somatic cell counts, received an energy-restricted diet (Bouvier-Muller et al., 2016, 2018). The authors showed that sheep from the low cell count line, which were less susceptible to mastitis, were also less susceptible to ketosis in early lactation (identified by measurements of concentrations of  $\beta$ -hydroxybutyrate and non-esterified fatty acids in blood). They concluded that these positive correlations suggested some commonalities in genetic control of immune response and energy metabolism, but may also reflect indirect associations due to competition for nutrients.

Finally, a question was put forward whether resistance to mastitis

was independent to resistance to other microbial diseases of sheep, e.g., footrot, paratuberculosis or *Small Ruminant Lentivirus* infection (Davies et al., 2009), but has not been addressed properly, due to lack of large-scale recording for those diseases.

### 3. Genomic basis for mastitis resistance

#### 3.1. QTL detection

##### 3.1.1. Primary discovery of QTLs

QTL for mastitis resistance have been evidenced in dairy sheep. Among primary QTL detection implemented within the ‘Genesheepsafety’ EU project, the common mastitis resistance phenotype was milk somatic cell count, measured periodically over a lactation period. Studies used low-density microsatellite panel (130 - 181 markers) at the time and only gave a limited resolution of the QTL locations and large chromosomal regions were not covered. One major finding was a QTL on *Ovis aries* chromosome 20 (OAR20), 30 cM distant from the MHC (Major Histocompatibility Complex) gene cluster, in a Churra population with 1421 ewes (Gutiérrez-Gil et al., 2007).

The availability of the Illumina OvineSNP50 beadchip, with 54 K SNPs in 2009 has allowed undertaking genome-wide association studies to map more precisely *loci* describing significant genetic variations in mastitis susceptibility. The ‘Sustainable Solutions for Small Ruminants’ (‘3SR’) project has been initiated, with the aim to implement genome scans for traits with high importance for health and sustainability in sheep, including mastitis resistance, and to develop selectable genetic markers and identify the causative mutation(s) where possible. QTL detection for somatic cell count was performed in three large dairy sheep populations by the consortium in France, Spain, and Italy (Sechi et al., 2013; Rupp et al., 2015; Gutiérrez-Gil et al., 2018). Those populations were as below.

- 2,414 individuals of a Sardinian back-cross population, including Back-cross ewes and 10 F1 sires (Sardinian × Lacaune), and Back-cross daughters with their sires (Sechi et al., 2013).
- 1,598 individuals from Churra daughters family population, including ewes and 16 sires (Gutiérrez-Gil et al., 2018).
- 1,009 artificial insemination sires from a Lacaune grand-daughter families, including sons from 33 sires (Rupp et al., 2015).

QTL detection was implemented using linkage analyses (LA), linkage analyses combined with linkage disequilibrium (LDLA) or linkage disequilibrium (LD) with various software and programs. Latest results from the three studies are summarised in Table 2. Genome-wide significant QTL regions associated with somatic cell count were found in 21 regions in the Churra population (Gutiérrez-Gil, 2013) by LDLA on OAR1, OAR2, OAR3, OAR8, OAR11, OAR13, OAR14, OAR17, OAR18, OAR19, OAR20, OAR22 and OAR25, of which the QTL on OAR20 and 25 were confirmed by linkage analysis. The linkage analysis method highlighted an additional QTL region on OAR5. The OAR20 region validated earliest results with microsatellites markers (Gutiérrez-Gil et al., 2007) and was selected for further fine mapping. Sechi et al. (2013) identified seven regions associated with somatic cell counts in the Sardinian back-cross population on OAR3 (two regions), OAR4, OAR5, OAR12, OAR19, OAR20. Further fine mapping for the most significant regions on OAR4 and OAR20 has been projected. Finally, genome-wide significant QTL regions associated with somatic cell count were found in five regions in the Lacaune grand-daughter population on OAR3, OAR4, OAR11, OAR16, OAR23 (Rupp et al., 2015). The QTL located on OAR3 exhibited a highly significant threshold and a narrow confidence interval (< 0.5 Mb) and was further studied.

##### 3.1.2. Fine mapping and identification of a causal mutation associated with mastitis

To fine map the most significant QTLs on OAR3 QTL in Lacaune

(Rupp et al., 2015) and OAR20 in Churra (Gutiérrez-Gil et al., 2018) ewes, authors subsequently used whole genome sequencing of three animals: a QTL sire and two of his sons with extreme phenotypes. Among candidate causal mutations, Gutiérrez-Gil et al. (2018) detected 13 variants distributed across seven immune-related genes predicted to cause an effect on protein function. The nominated genes were: *PKHD1*, *NOTCH4*, *AGER*, *MOG* and three genes orthologous to the human MHC: *ENSOARG0000009395* (*HLA-C*, *Homo sapiens*), *ENSOARG00000015002* (*HLA-B*, *Homo sapiens*) and *ENSOARG00000018075* (*BoLA*, *Bos taurus*, orthologous to human *HLA-A*).

For the OAR3 region, Rupp et al. (2015) identified a candidate SNP mutation that mapped to the coding sequence of the highly conserved gene *suppressor of cytokine signalling2* (*SOCS2*). An assay for evaluating the protein binding affinity suggested a functional knockout of the *SOCS2* gene in homozygous susceptible sheep. The authors proposed that this mutation altered actual function in the JAK/STAT/SOCS pathway and the control of the inflammatory response to infection. Genetic variants in genes involved in the JAK/STAT/SOCS pathway (*STAT5A* and *JAK2*) were previously associated with mastitis indicator traits (SCC) in Chinese Holstein cattle (Usman et al., 2014), which also supported the importance of this pathway in the determinism of the host’s response to mastitis. It has to be noted that Rupp et al. (2015) have further reported a pleiotropic favorable effect of the *SOCS2* mutation on body growth and milk production. This has provided a molecular basis for the antagonism between mastitis resistance and production traits and has highlighted the need for better knowledge on such genetic variants with adverse effects to achieve optimal balancing selection.

##### 3.1.3. Validation of QTLs in independent populations

Generally, QTLs had moderate effects; for the most significant, they explained 3% of the variance for the Churra OAR20 QTL and up to 12% for the Lacaune OAR3 QTL. The QTLs were mostly population specific, in agreement with the polygenic nature of the trait and the complexity of the resistance phenotype. When considering also less significant QTLs though, a few commonalities among populations have been identified, e.g., on OAR12 and OAR20 (Table 2). Accordingly, the 3SR consortium partners designed a public custom-designed ovine SNP chip ([http://genoweb.toulouse.inra.fr/~tossier/3SR-WP3-960\\_snp\\_mastitis/](http://genoweb.toulouse.inra.fr/~tossier/3SR-WP3-960_snp_mastitis/)) for validating the most significant QTL and the common QTL regions in independent populations at the time. The validation chip comprised 960 SNPs distributed over seven genomic regions. SNPs were selected from the OvineSNP50 beadchip or 800 K Illumina ovine chips (Nicolazzi et al., 2015) or were identified within the ‘3SR’ project by genome resequencing.

Validation studies were performed using this custom chip in three independent dairy sheep populations: Chios (Banos et al., 2017), Lacaune and Manech Red Face ewes (Oget et al., 2019). Banos et al. (2017) confirmed significant QTL associated with four different mastitis related-traits on OAR2, OAR3, OAR5, OAR16 and OAR19; they also highlighted 14 relevant candidate genes implicated in innate immunity in these regions, based on several analyses such as a pathway and functional clustering analysis, a gene expression analysis and a transcription factor binding site analysis. The selected genes included *SOCS2*, *CTLA4*, *C6*, *C7*, *C9*, *PTGER4*, *DAB2*, *CARD6*, *OSMR*, *PLXNC1*, *IDH1*, *ICOS*, *FYB* and *LYFR* (Banos et al., 2017). Oget et al. (2019) validated four of the seven mastitis QTL regions: OAR2 (Lacaune and Manech Red Face), OAR3 (Lacaune), OAR16 (Manech Red Face), OAR19 (Lacaune). The point mutation in *SOCS2* (OAR3 QTL region), included in the 960 SNP chip, was the most significant SNP associated with three mastitis related-traits: increased SCS, increased incidence of bacterial infection and mammary abscess. This mutation was also significantly associated with increased body growth, confirming the pleiotropic effect of the *SOCS2* gene. This SNP was not present in the Manech Red Face available data. Two validated regions (OAR2 and 16) were also associated with milk production traits in both populations,

**Table 2**

Significant QTL influencing somatic cell scores identified by the Combined Linkage Disequilibrium and Linkage Analysis (LDLA), Linkage Disequilibrium (LD) or Linkage Analysis (LA) in three dairy sheep populations.

OAR	Position (Mb)	Confidence interval	Method	Sheep breed	Reference
1	259.05	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
2	83.10	83.1 - 83.2	LDLA	Churra	Gutiérrez-Gil et al., 2018
	140.36	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
3	185.00	185.0 - 185.1	LDLA	Churra	Gutiérrez-Gil et al., 2018
	4.40	4.3 - 4.4	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
	22.65	22.6 - 22.8	LD	Lacaune	Rupp et al., 2015
	94.40	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	129.94	129.8 - 130.0	LD	Lacaune	Rupp et al., 2015
	130.10	129.4 - 131.4	LA	Lacaune	Rupp et al., 2015
	184.40	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	189.57	189.6 - 189.6	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
	212.70	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	4	15.86	15.8 - 16.0	LD	Lacaune
46.99		42.8 - 56.7	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
5	68.69	68.6 - 68.9	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
	77.99	74.9 - 81.5	LA	Churra	Gutiérrez-Gil et al., 2018
	102.69	102.6 - 102.8	LD	Lacaune	Rupp et al., 2015
6	71.31	71.1 - 71.5	LD	Lacaune	Rupp et al., 2015
7	24.53	24.4 - 24.6	LD	Lacaune	Rupp et al., 2015
8	59.85	59.7 - 59.9	LD	Lacaune	Rupp et al., 2015
	80.60	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
9	82.60	81.4 - 83.5	LA	Lacaune	Rupp et al., 2015
	27.49	27.4 - 27.6	LD	Lacaune	Rupp et al., 2015
10	49.30	48.9 - 49.6	LA	Lacaune	Rupp et al., 2015
	74.84	74.7 - 74.9	LD	Lacaune	Rupp et al., 2015
11	36.70	35.8 - 41.1	LA	Lacaune	Rupp et al., 2015
	41.18	41.0 - 41.3	LD	Lacaune	Rupp et al., 2015
12	56.78	56.8 - 56.9	LDLA	Churra	Gutiérrez-Gil et al., 2018
	13.84	13.7 - 13.9	LD	Lacaune	Rupp et al., 2015
13	18.43	9.7 - 18.4	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
	70.89	70.8 - 71.1	LD	Lacaune	Rupp et al., 2015
14	71.82	68.5 - 71.8	LDLA	Churra	Gutiérrez-Gil et al., 2018
	32.00	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
16	39.40	36.4 - 40.2	LA	Lacaune	Rupp et al., 2015
	43.28	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	56.58	56.5 - 56.7	LD	Lacaune	Rupp et al., 2015
	5.89	5.8 - 6.0	LD	Lacaune	Rupp et al., 2015
17	36.10	35.2 - 37.0	LA	Lacaune	Rupp et al., 2015
	23.65	23.5 - 23.7	LD	Lacaune	Rupp et al., 2015
18	33.80	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	42.30	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
	12.65	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
19	31.18	30.9 - 31.3	LD	Lacaune	Rupp et al., 2015
	26.17	–	LDLA	Churra	Gutiérrez-Gil et al., 2018
20	28.60	28.4-28.7	LD	Lacaune	Rupp et al., 2015
	56.70	56.7-56.78	LDLA	Sarda, Sarda × Lacaune	Sechi et al., 2013
	5.50	5.4-5.5	LDLA	Churra	Gutiérrez-Gil et al., 2018
	21.52	20.9-23.8	LA	Churra	Gutiérrez-Gil et al., 2018
22	23.52	22.0-28.0	LDLA	Churra	Gutiérrez-Gil et al., 2018
	23.75	13.8-25.4	LDLA	Churra	Gutiérrez-Gil et al., 2018
	48.73	48.6-48.8	LD	Sarda, Sarda × Lacaune	Sechi et al., 2013
	23.76	–	LDLA	Lacaune	Rupp et al., 2015
23	48.47	48.4-48.6	LD	Churra	Gutiérrez-Gil et al., 2018
	59.94	59.8-60.0	LD	Lacaune	Rupp et al., 2015
24	73.00	7.2-7.4	LD	Lacaune	Rupp et al., 2015
25	16.58	15.6-16.6	LDLA	Churra	Gutiérrez-Gil et al., 2018
	32.50	32.5-35.0	LDLA	Churra	Gutiérrez-Gil et al., 2018
	39.48	38.6-41.7	LA	Churra	Gutiérrez-Gil et al., 2018
26	19.57	19.5-19.7	LD	Lacaune	Rupp et al., 2015

indicating, at least in part, a genomic basis for the trade-off between milk production and mastitis resistance. These validation studies testify to the partial sharing of mastitis-related genetic mechanisms between different distant dairy sheep populations.

### 3.2. The input on mechanisms from gene expression data

A number of studies have investigated the host response to mastitis, using gene expression data. The studies were performed on various cell types: milk somatic cells (Bonnetfont et al., 2011), dendritic cells

(Genini et al., 2011; Toufeer et al., 2011), mammary epithelial cells (Bonnetfont et al., 2012) or mammary tissue samples (Chopra-Dewasthaly et al., 2017). Cells were exposed either to a microbial agent (*Staphylococcus aureus*, *S. epidermidis*, *Staphylococcus* spp., *Mycoplasma agalactiae*) in vivo after an intramammary challenge or to inactivated pathogens or bacterial soluble factors in vitro in cultured cells. Up to date, microarrays have mostly been used, either with ovine oligonucleotide (homologous) probes (Bonnetfont et al., 2011; 2012; Toufeer et al., 2011) or bovine cDNA (heterologous) probes (Genini et al., 2011). One publication thus far has used RNAseq profiling (Chopra-

Dewasthaly et al., 2017), but it can be anticipated, however, that future studies will use RNASeq analysis, since it gives a more exhaustive view of the transcriptome. In all studies, but the RNAseq profiling, Lacaune ewes from a divergent selection experiment based on somatic cell were used. The main outcomes of these studies were as below.

- Larger T-cell recruitment after *S. aureus* as compared to *S. epidermidis* challenge was evident.
- A significant difference was noted in the gene expression profile of milk somatic cells and dendritic cells in the genetic divergent lines, suggesting that they played a major role in genetic resistance/susceptibility, whereas mammary epithelial cells exhibited a more similar profile.
- Milk somatic cells gene profiling showed several differences between animals from the two divergent lines, suggesting a relationship between immune response and genetic resistance; this included cytokine and chemokine differential expression, a potentially more efficient neutrophil diapedesis, a more efficient clearance of the infection through TLR2 and MAPK signaling pathways, a limitation of cell proliferation and apoptosis in the resistant line.
- In dendritic cells, resistant animals showed upregulation of complement pathway genes and downregulation of the IL1R1 pathway.
- Transcriptome differences between resistant and susceptible lines were related to transcriptional activity within Milk Somatic Cells. RAR $\alpha$ , TP53, AHR were transcriptional factors of interest, both because of their differential expression and their interaction with a larger number of regulated genes between lines.

Interestingly, Banos et al. (2017) used crossed information from QTL detection and several public and in house datasets to propose 14 candidate genes for mastitis. This was based on the assumption that genes contributing to mastitis resistance are likely to be expressed both in the mammary gland and in immune tissues and differentially expressed in sheep bone marrow-derived macrophages in response to lipopolysaccharide administration. This analysis was completed with the study of 1 kb upstream regions of the Transcription Factor Binding Sites different in dairy and meat breeds. Further studies are required to confirm the functional impact of the candidate polymorphisms.

#### 4. Breeding for improved mastitis resistance

##### 4.1. Selection criteria and breeding objectives

Breeding programs in dairy sheep were first developed in the 1960s. Only a few dairy populations worldwide, mainly located in the para-Mediterranean region (Lacaune, Manech, Basco-béarnaise [France], Assaf, Latxa, Manchega, Churra [Spain], Sarda [Italy], Chios [Greece] populations) have the required size and organisation to allow development of large scale recording, genetic evaluation and breeding programmes (Barillet et al., 2001; Carta et al., 2009; Gootwine, 2011; Theodoridis et al., 2018). Milk yield has been the main selection criterion in most of those breeds during the past decades (Carta et al., 2009). Thereafter, in some breeds, a component for milk composition (Barillet et al., 2001; Gutiérrez-Gil et al., 2009) and udder morphology (Casu et al., 2006; Marie-Etancelin et al., 2006) has been added.

Following the cattle initiative in the 1980s (Heringstad et al., 2000), in several dairy sheep populations, milk somatic cell counting has been considered as a proxy for mastitis and included that in recording schemes and breeding objectives. Thus, somatic cell counting recording has been implemented in several breeds in France (Lacaune, Manech), Italy (Sarda) and Spain (Churra, Manchega, and Laxta) (Carta et al., 2009). Repeated cell counting data can be routinely recorded as part of milk recording. However, the recording cost per animal, relative to the potential income, is prohibitive for many traits other than production. Therefore, simplified schemes have been developed, with only 2–4 test days per lactation period or with sampling only in ewes in their first or/

and second parity (Rupp et al., 2002; Carta et al., 2009).

Up to date, systematic selection for somatic cell counting in addition to milk production and udder morphology is being performed only in Lacaune sheep (Rupp et al., 2002; Barillet et al., 2009). In this breed, the current relative weight for somatic cell count is 25% in the total merit index, with relative weights of somatic cell scores compared to the production of 1:2. At current selection intensities, such a combination is expected to reduce somatic cell scores by one genetic standard deviation in 10 years. Recently, a genetic evaluation for somatic cell count has been initiated in Manech Red Face sheep, updating accordingly the breeding objectives. Indirect selection for improved mastitis resistance can be also expected if udder morphology is used for selection, as this gives favorable genetic correlations between traits, as performed in Sarda sheep (Casu et al., 2006).

A divergent selection experiment based on somatic cell scores in Lacaune dairy sheep has provided evidence that selection for decreased milk somatic cell count may help to improve host's resistance to mastitis and decrease the frequency for clinical and subclinical intramammary infections (Rupp et al., 2009; Allain et al., 2010). Based on the results of approx. 200 ewes in each line selected for either high or low somatic cell scores, authors have shown a large difference between the genetic lines for somatic cell count: mean cell counts were 1,200,000 versus 280,000 cells mL<sup>-1</sup>, respectively. A significant decrease of clinical mastitis, chronic clinical mastitis (as detected by the presence of parenchymal abscesses), and intramammary infections caused by various pathogens (measured by repeated milk bacteriological tests) was also observed in the low somatic cell score line when compared to the high line (Rupp et al., 2009; Allain et al., 2010). The reduced risk of mastitis when selecting for decreased somatic cell scores was further confirmed in two experiments, in which ewes from the divergent lines were challenged with *S. epidermidis* or *S. aureus* (Bonfont et al., 2011). The results indicated that selection for decreased somatic cell scores correlated with a better ability to control intramammary multiplication of bacteria and to limit consequences of infection and inflammation.

Minor consideration has been given to mastitis in meat sheep industry up to date. However, in a meat breed (Texel), Conington et al. (2008) have highlighted economic and welfare benefits through the reduction of antibiotic use and extra labor involved. These authors estimated that a 10% reduction in risk of developing mastitis would be leading to GBP 8.40 per ewe, or GBP 2.7 million a year to the purebred Texel population in the United Kingdom (Conington et al., 2008; McLaren et al., 2018). Additionally, the authors underlined that increased incidence of subclinical mastitis had an adverse in the body weight of lambs of affected ewes and thus in overall production output of the flock. However, given that in meat-production flocks ewes are not handled on a daily basis, as in dairy flocks, use of somatic cell count poses challenges. In view of that, McLaren et al. (2018) have suggested results of California Mastitis Test and udder conformation scoring could be useful alternative selection criteria for future genetic selection.

##### 4.2. The contribution of genomics to breeding for mastitis resistance

###### 4.2.1. Direct use of information from major genes

Diagnostic tools can be developed for genotyping major genes, when causal mutation or closely linked markers, are identified. As an example of a disease-related gene, testing for *PrP* gene associated to resistance and susceptibility to scrapie (Elsen et al., 1999), is used in breeding programs worldwide, mainly to avoid entering susceptible animals in the reproduction process. Other significant genes in sheep are *Tmem154*, associated with resistance to *Small Ruminant Lentivirus* infections (Heaton et al., 2012), and *FecL* for hyperprolificacy of ewes (Martin et al., 2014). To date, the large-scale development of such marker-assisted selection for improving mastitis resistance in various sheep populations is unlikely. Indeed, the *SOCS2* gene is the only published causal mutation for mastitis resistance and the susceptible

allele was found only in the Lacaune breed (Rupp et al., 2015).

Mastitis resistance is highly polygenic with a large number of genes with small effects, which is not well suited for single gene-based selection. With the decrease of sequencing and genotyping costs and the addition of genomic studies in sheep, it is expected, though, that additional causal mutations will be available in the near future. One interesting opportunity is the development of small sets of parentage SNP or low-density chips including also major gene information, which could allow extending such diagnosis approach for various traits and population at reasonable costs.

#### 4.2.2. Genomic selection

The objective of genomic evaluations, called by extension ‘genomic selection’, is to estimate the genetic value (called Estimated Breeding Value, i.e., EBV) of an individual for a given trait (e.g., mastitis resistance, i.e. somatic cell counts) based on genomic information, such as SNP markers covering the whole genome (Meuwissen et al., 2001). No information on the genes or genomic regions associated with the trait is necessary. Genomic evaluation requires a significant number of genotyped and phenotyped individuals, forming the ‘reference population’, in which associations between SNP and phenotypes are established. Then, using the established predictions, genomic EBVs are estimated in a population of related candidates, for which no phenotype is (yet) available. The benefit from the genomic selection is derived from the fact that genomic EBVs have higher accuracies, i.e. better predict future achieved performance, than pedigree-based EBVs, so that early selection can be efficient. Thus, genomic selection can be useful in sheep breeding to increase the genetic gain by decreasing the generation interval. This is especially true in dairy sheep because there is a long period between the dissemination of male’s semen across the population and collection of phenotypes recorded in female progeny. Genomic selection in ruminants can also be useful for traits that are not measured in large-scale population as a routine, e.g., mastitis in meat sheep breeds.

A few studies have compared pedigree-based selection and selection based on genomic information for mastitis resistance (SCS trait) in the Lacaune dairy breed and have reported a gain of prediction accuracy ranging from +0.04 to +0.10 (Duchemin et al., 2012; Baloche et al., 2014). These results are lower than in cattle (+0.15) (VanRaden et al., 2009) probably mainly because reference population is smaller and the genetic diversity is higher, as proven by lower linkage disequilibrium (Baloche et al., 2014). Currently, the so-called Single Step Genomic Best Linear Unbiased Prediction (ssGBLUP) method provides the best accuracies in dairy sheep (Baloche et al., 2014); the model uses raw performances of daughters rather than ram’s averages.

Future improvement of genomic selection is expected from the inclusion of major genes and QTLs in genomic evaluation models. Indeed, knowledge of genes/genomic regions with a strong effect can be added to the genome-wide SNP panel or added independently as a correlated trait, like in the Gene Content Multiple trait BLUP model (Legarra and Vitezica, 2015). Such approaches have proved to increase prediction accuracy for goat milk composition, with the inclusion of information on the  $\alpha$  s1 casein (Carillier-Jacquin et al., 2016; Teissier et al., 2018) major genes together with the 50 K SNP chip. Application to mastitis resistance, with the inclusion of the *SOCS2* gene or other QTLs, is promising.

## 5. Concluding remarks

In this paper, we summarized recent advances in genetic and genomic analyses for the improvement of mastitis resistance in dairy and meat-production sheep. The numerous studies on the correlations between the different traits of interest in these two sheep sectors, as well as the major new genes discovered thanks to the progress of genomic tools, pave the way for a selection of more robust individuals for sustainable breeding.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.smallrumres.2019.05.011>.

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